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Review

Research Progress of Plant Nucleotide-Binding Leucine-Rich Repeat Protein

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Abstract: Nucleotide-binding leucine-rich repeat sequence (NBS-LRR) protein is the main immune receptor in plants and participates in plant resistance to pathogens. When the NBS-LRR protein is activated by the pathogen's effector protein, its conformation changes from an inhibitory state to an activated state, then it activates downstream signal transduction and initiates defense responses to inhibit the growth of pathogens. The NBS-LRR protein has major three domains: NBS, LRR and TIR/CC, which all play a certain role in the immune response induced by it. In this paper, the NBS-LRR protein domains and their functions, molecular mechanism of the induced immune response and its application in disease resistance breeding are reviewed.

Keywords: plant; NBS-LRR protein; signal transduction; immune response; disease resistance breeding

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1. Introduction

In plants, numerous potential pathogens (including viruses, bacteria, fungi and nematodes) may cause severe declines in crop yield and quality [1]. In order to recognize and defend against pathogen infection, plants form the innate immune system. The innate immune system of the plant has two layers: the first layer of immunity is PAMP-triggered immunity (PTI) when the extracellular pattern-recognition receptors (PRR) of the host cytoplasmic membrane interacts with pathogen-associated molecular patterns (PAMPs); the second level of immunity is the identification of the pathogenic factor effector in the pathogen, which activates the effector-triggered immunity (ETI). The identification at this level will activate a stronger reaction, which is typically programmed cell death. Plant resistance gene (R gene) plays a key role in this progress [2]. NBS-LRR (Nucleotide tide Binding Site-Leucine Rich Repeat) gene is the main gene of R genes. NBS-LRR protein encoded by NBS-LRR gene is the main immune receptor of plants. When it is activated by the effector protein of pathogens, NBS-LRR protein starts the defense response, such as the explosion of active oxygen, hypersensitive response (HR), to inhibit the growth of pathogens [3].

2. Plant NBS-LRR Protein

NBS-LRR protein is a resistance protein encoded by NBS-LRR gene in plants, which helps plants resist the invasion of pathogens. NBS-LRR gene is abundant in plants, such as *Arabidopsis thaliana* and soybean, which contain 149 and 319 NBS-LRR family genes, separately. Rice genome contains about 500 NBS-LRR family genes. 267 NBS-LRR family genes are identified in sugar beet genome, 138 NBS-LRR family genes are identified in cabbage genome. 411 NBS-LRR family genes in all are obtained in *Setaria italica* [4–8]. NBS-LRR protein is also called NB-ARC-LRR (nucleotide binding adaptors composed of Apaf-1, R protein and CED-4) protein because it contains nucleotide binding and ATPase domain (NB-ARC), which can be a signal transduction domain with multiple domain

protein superfamily. The conserved domain in ATPase can be used as a switch to regulate protein activation [9].

3. Domain and Function of Plant NBS-LRR Protein

Plant NBS-LRR protein mainly has three domains, which are the central NBS domain, leucine-rich repeats (LRR) domain, N-terminal coiled -coil (CC) domain or TIR domain (Figure 1).

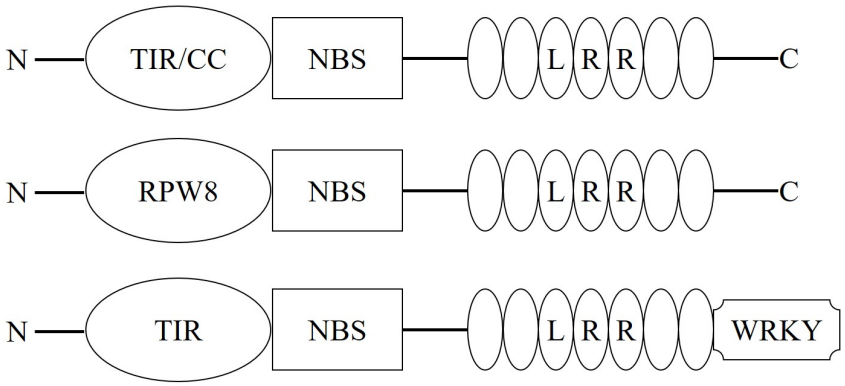

Figure 1. Domain of NBS-LRR protein in plants.

3.1. NBS Domain

NBS is a kind of conserved domain which has an N-terminal domain. It may contribute to disease-resistant signal transduction and play a vital role in plants response to pathogen invasion. NBS domain is constituted by a number of conserved motifs, like P loop (kinase 1a), kinase 2, kinase 3a and transmembrane domain GLPL [10]. P loop motif with common sequence GXXXXGK(T/S) participates in binding to phosphate and Mg21 ions. Kinase 2 is quite vital in phosphate transfer reaction. It is composed of four continuous hydrophobic amino acids and conserved aspartic acid (Asp) residues, which coordinate divalent metal ions on Mg-ATP. Kinase 3a motif participates in purine or ribose binding and comprises conserved tyrosine (Tyr) or argimine (Arg) residues [10]. NBS domain can cause a conformational transition, from a concentrated ADP-bound state to an open ATP-binding state with exposed N-terminal domain, it further stimulates downstream HR reaction, cause apoptosis of infected cells. It eventually inhibits the proliferation and diffusion of pathogens [11]. NBS domain can also participate in cell growth, differentiation, cytoskeleton, vesicle transport and defense. In addition, NBS domain can also participate in the oligomerization process of N- terminal signal domain.

The conserved sequences in NBS domain can be used to identify new disease resistance genes in model plants and various crops. Using it to design degenerate primers to clone the resistance gene analogues (RGAs) is a method to obtain new disease resistance genes. At present, the disease resistance genes of Amorphophallus konjac and other plants have been obtained by this method [12]. It provides a guarantee for the follow-up study on the disease resistance mechanism of NBS-LRR gene in other plants.

3.2. LRR Domain

LRR is a continuous and repeated leucine-rich amino acid sequence with a motif length of 20–29 residues, and it is made up by a conserved 11-residue fragment LxxLxLxxN/cxL (X can be any amino acid, and L can be replaced by valine (Val), isoleucine (Ile) and phenylalanine (Phe)) [13]. α-helix and β-fold constitute its spatial structure. Protein containing LRR plays an important role in hormone receptor interaction, enzyme inhibition, cell adhesion and cell transport [14]. Studies have shown that LRR domain provides a general structural framework for the formation of protein-protein interaction. LRR domain is the main factor of specific resistance of NBS-LRR gene.

3.3. TIR/CC Domain

The TIR domain contains about 175 amino acids, including the 3′ frame of conserved residues in the central sequence of 135 to 160 amino acids and two interfaces that mediate the interaction of TIR domains. TIR domain is involved in specific resistance and signal transduction. For example, in the innate immune response of plants to bacteria and fungi, Toll-like receptors (TLRs) and IL-1R signal pathway are the key to this response [15]. In addition, the interaction of TIR domain between receptor and adapter plays a key role in activating the conserved cell signal transduction pathways that respond to bacterial Lipopolysaccharides (LPS), microbial and viral pathogens, cytokines and growth factors. TIR-type proteins are usually found in dicotyledons, but few or no proteins are found in monocotyledons.

CC domain, as the oligomerization domain of various protein, is composed of three parts: structural protein, movement protein and transcription factor. CC domain usually consists of two or more α-helices, which are intertwined with each other in the form of supercoils. The α-helix structure consists of seven residue repeat sequence (abcdefg) n, in which a and d represent hydrophobic residues, and e and g are polar residues. CC domain is the key to protein-protein interaction. For example, a CNL protein Rx of potato interacts with the coat protein of potato virus X (PVX) to give potato resistance to PVX [16]. CC-type proteins are found in dicotyledons and monocotyledons.

In addition, the N-terminal NBS-LRR protein of some angiosperms contains a domain of resistance to powdery mildew 8 (RPW8), which is crucial in the transduction of immune signals [17]. This NBS-LRR protein is called RNL, which have two lineages: ADR1 and NRG1. What is found is that Arabidopsis NBS-LRR protein RRS1 also contains a WRKY domain (zinc-finger transcription factor-related domain containing the WRKY sequence) (Figure 1), and the gene encoding it can mediate the resistance of *Arabidopsis thaliana* to *Ralstonia solanacearum* [18].

4. Molecular Mechanism of Immune Response Induced by Plant NBS-LRR Protein

4.1. Immune Response Induced by Plant NBS-LRR Protein after Recognizing Pathogen Effector Protein

NBS-LRR protein is sensitive to pathogen effector protein, which causes effector protein to trigger immune response ETI, so that plants can produce defensive response against bacteria, viruses, fungi and other pathogens. NBS-LRR protein not only is able to directly or indirectly recognize effector protein products of pathogens, but it can also interact with them [19]. This recognition of effector can lead to the conformation change of NBS-LRR protein, it changes from the inhibition state to the activation state, thus activating the downstream signal transduction and generating the defense response.

The immune response induced by NBS-LRR protein after recognizing pathogen effector protein is usually related to its localization in cells (Figure 2). The effector protein PopP2 of *Ralstonia solanacearum* is sensed by RRS1-R resistant protein in *Arabidopsis thaliana* and triggers immunity in the nucleus. PopP2 can prevent the proteasome degradation of RRS1-R [20]. With the powdery mildew effector protein AvrA10, barley CNL protein MLA10 will migrate from cytoplasm to nucleus and interact with WRKY (transcription repressor) and MYB6 (transcriptional activator) to make the defense response activate [21]. The TNL protein RPS4 of *Arabidopsis thaliana* can be transferred to the nucleus when recognizing AvrRps4, thus interacting with the CNL protein RRS1 of *Arabidopsis thaliana* to activate the defense response. Meanwhile, a subset of RPS4 complex remains in cytoplasm to activate HR [22]. NBS-LRR protein can activate resistance not only in nucleus, but also in cytoplasm and plasma membrane. After recognizing potato virus X coat protein (PVX CP), potato CNL protein Rx interacts with cytoplasmic activator RanGAP2 to activate signal pathway. However, the recognition and signal activation of PVX CP occurs in nucleus when cytoplasm PVX CP accumulates, Rx will not be activated, but accumulation in cytoplasm will enhance the function of Rx [23]. Likewise, three Arabidopsis CNL proteins RPS5, RPS2 and RPM1 need to recognize the corresponding pathogen effectors in plasma

membrane and interact with protein kinases and activators to function [24,25]. In addition, the recognition and signal initiation of potato resistance protein R3a to AVR3a (KI) occur in the endocytosis vesicle.

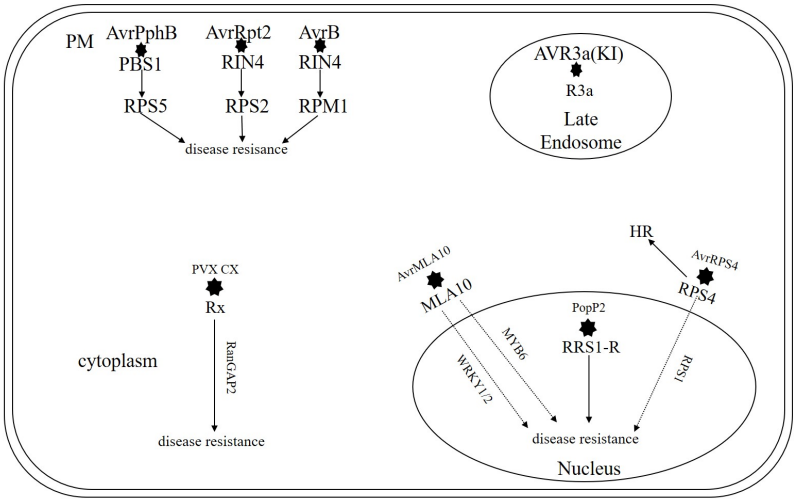

Figure 2. Diversified localization of plant NBS-LRR protein (according to Dong Qi).

4.2. Immune Response Induced by Interaction between Plant NBS-LRR Protein and Molecules in Signal Transduction Process

There are complex networks of distinct signal transduction in the interaction between plant pathogens. The domain of NBS-LRR protein is very significant in inducing immune response, because it is able to interact with downstream signal components. In a key signal pathway, a small GTPase OsRac1 interacts with the CC domain of rice NBS-LRR protein Pit, and is activated by Pit, which induces the generation of reactive oxygen and HR response and helps Pit resist the invasion of *Magnaporthe oryzae* [26]. Zhou et al. [27] found that the NBS domain of rice NBS-LRR protein PID3 interacted with OsRac1 to induce the expression of transcription activator RAI1, thus resisting the invasion of *Magnaporthe oryzae*. Because of the NB domain of PM2b and the WRKY domain of TaWRKY76-D, they interact and regulate the resistance of common wheat to powdery mildew [28]. The CC domain of rice NBS-LRR protein Pit interacts with DOCK guanine nucleotide exchange factor (GEF) protein OsSPK1 to activate OsRac1 on plasma membrane, thus inducing Pit-mediated immune response. OsSPK1 is the direct and critical signal transduction target of Pit-mediated immunity [29]. The CC domain of rice NBS-LRR protein Pik-H4 interacts with OsBIHD1, through the coordination of ethylene (ET)-brassinosteroid (BR) to regulate the balance between rice blast resistance and growth [30]. The interaction between the NBS-LRR and WRKY protein is possibly to expedite the activation of R genes in *V. Unguiculata* and the production of protease inhibitor enzymes acts to block the development of *M. incognita*. The maintenance of actin cytoskeleton appears to be necessary in cowpea resistance, preventing giant cell formation through cytoskeleton remodeling caused by *M. incognita* [31]. Under the invasion of *Magnaporthe oryzae*, SH3P2 will first combine with AvrPib, and then interact with the CC domain of Pib, changing from SH3P2-Pib heterodimer to Pib homodimer, and then triggering ETI [32]. Two RNL lineages (ADR1and NRG1) can induce TNL-triggered immunity. The EDS1–SAG101 and EDS1–PAD4 complexes can further interact with NRG1 and ADR1 significantly, which is able to form the EDS1–SAG101–NRG1 and EDS1–PAD4–ADR1 modules to transmit resistance signals [33,34].

4.3. Other Ways of Plant NBS-LRR Protein Inducing Immune Response

Gene expression regulation of NBS-LRR protein is not only through direct interaction with transcription factors to generate resistance, but also induces immune response through other ways. Nematode effectors can be recognized by NBS-LRR, and ETI is activated by the

signal cascade of defense gene activation [35]. Reverse genetics also found that the resistance mediated by RPS2 and RPM1 at least partly depended on calcium-dependent protein kinases (CPKS) [36]. CPK1 and CPK2 contribute to the generation of HR reaction, and CPK1/2/4/11 also promotes the generation of reactive oxygen by phosphorylation with NADPH oxidase. To sum up, when NBS-LRR is activated, it triggers the continuous inflow of calcium, and then triggers multiple CPK signal pathways, leading to ROS generation, defense gene activation and cell death. Studies have also shown that secretion may take part in NBS-LRR-mediated defense. RPS2 plays an up-regulation role in the transcription of miR393b, which is a microRNA and has three target genes: MEMB12 (which codes for the Golgi apparatus-located SNARE protein), *VPS54* (which codes for the homologous yeast protein involved in retrograde transport from secondary endosome to Golgi apparatus), and *EXO70H3* (the subunit of vesicle complex necessary for exocytosis), all three genes are involved in the membrane transport process. Knocking out *MEMB12* can also enhance the secretion of defense protein PR-1 induced by activation of RPS2 [37]. MiR1510 plays a negative regulatory effect in the resistance of soybeans *Phytophthora sojaeby* by inhibiting NBS-LRR gene *GmTNL16* expression. JA and SA participate in *GmTNL16*-mediated *P. Sojae* resistance in soybean [38]. GauCNL18 can activate the salicylic acid signaling pathway which mediate up-regulated pathogenesis-related genes and reactive oxygen species accumulation to enhance *Verticillium dahlia* resistance [39].

5. Application of Plant NBS-LRR Protein in Disease-Resistant Breeding

To prevent the invasion of pathogens and activate defense mechanisms, plants sense pathogen effector proteins through NBS-LRR proteins in cells, which leads to ETI as a method of defense. NBS-LRR genes in distinct plants can directly or indirectly endow plants with resistance and help them resist the attack of pathogens. For instance, the wheat NBS-LRR gene *Pm60* gives wheat resistance to powdery mildew [40]; the wild eggplant NBS-LRR gene *SacMi* is involved in the resistance of plants to *Meloidogyne spp* [41]; The expression of NBS-LRR gene in tomato is positively correlated with the resistance of plants to *Phytophthora infestans* [42].

At present, the best measure to control diseases in agricultural production is the application of resistant varieties. NBS-LRR gene is widely used in molecular breeding. It was found that the transgenic plants obtained by transferring wheat NBS-LRR gene *TaRCR1* into wheat varieties showed obvious resistance to *Rhizoctonia cerealis* [43]. Over-expression of NBS-LRR gene *AhRRS5* in peanut improved tobacco resistance to bacterial wilt [44]. *RppM*, encoding a typical CC-NBS-LRR protein, confers resistance to southern corn rust in maize and it has great potential utility in the cultivation of durable resistant maize cultivars [45]. A NBS-LRR gene *Rps11* confers broad-spectrum resistance to *Phytophthora sojae* in soybean. The unique structural features of Rps11 make this NLR a suitable model for resistance enhancement [46]. Maize NBS-LRR gene *ZmNBS25* improved the disease resistance of rice [47]. GmTNL16 belongs to TIN-NBS-LRR type protein, and the immune response to Phytophthora root rot can be regulated by it [38]. The *Sm* gene encodes a tomato NBS-LRR plant resistance protein, and is able to confer resistance to gray leaf spot disease [48].

6. Outlook

NBS-LRR protein is the key to the interaction between plants and pathogens. With the application of genome-wide technology and high-throughput sequencing technology, more and more NBS-LRR genes encoding NBS-LRR protein have been identified, thus revealing their role in resisting various pathogenic diseases. For example, genome-wide analysis of NBS-LRR gene of *Xanthomonas oryzae pv. Oryzae* (Xoo) in rice is helpful to revealing the expression and regulation of NBS-LRR gene in rice resistance to Xoo [49]. Genome-wide analysis of NBS-LRR gene *Lagenaria siceraria* is helpful to reveal the expression and regulation of NBS-LRR gene resistance to powdery mildew [50]. In addition, the molecular

mechanism related to NBS-LRR protein-induced immune response has also been studied, but the signal transduction process of the whole disease resistance pathway is not clear yet.

At present, there are few studies on fungal pathogens that state how NBS-LRR protein participates in the regulation of plant resistance response. In order to explain the interaction between fungal pathogens and host plants, another defense mechanism, called Effector triggered defence (ETD), was summarized by Stotze et al. [51]. Compared with ETI, ETD has a relatively slow response to pathogens, rather than the rapid allergic cell death reaction. In some pathogenic systems of plant necrotic fungi, the recognition of NBS-LRR protein to effector proteins produced by pathogens leads to the susceptibility to trigger of effector proteins, but the specific processes of this defense mechanism are still unclear.

Breeding disease-resistant varieties is an important method to help plants resist pathogens. Stable genetic transformation has been used to transfer disease-resistant genes into plants to obtain disease-resistant plants. At present, the application of NBS-LRR gene encoding NBS-LRR protein in disease-resistant breeding is about heterologous expression, while the application of homologous expression is almost in model plants. Therefore, it is very important to improve the application of stable genetic transformation technology in plants. Gene editing based on CRISPR Cas is a powerful genetic engineering method and a valuable tool for research and breeding. The application of disease-resistant breeding also has disadvantages. Resistance genes are highly specific and only effective for one pathogen, but plants will be invaded by multiple pathogens. In addition, breeding disease-resistant varieties also requires metabolic costs. Consequently, solving these problems will be the focus of follow-up study. For example, multiple resistance genes can be aggregated into the same genotype, or using genes related to durable resistance, stable genetic transformation technology should be optimized. This will be the top priority in the follow-up study of NBS-LRR protein.

Many beneficial soil microorganisms in root microbiota can enhance the resistance of plants to stress. Some chemical pesticides can also help plants resist pathogens. However, these methods also have some disadvantages. As Zhang said, an integrated approach that combines genetic, chemical, and microbial strategies is the best, and it can provide solutions for cultivating plants with high stress resistance and productivity. This is also the focus of future research [52].

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